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Target validation

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1 Target Validation

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3 News & Views

4 5 Switching domains

6
7 **Chemical probes and drugs often bind to functional domains on disease-relevant proteins. A**
8 **study suggests a chemical genetic approach to establish on-target effects by swapping the**
9 **targeted domain, affords resistance to pharmacological inhibition while retaining**
10 **functionality.**

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12 Alessio Ciulli

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14 In target-driven discovery world, a founded paradigm of chemical intervention involves
15 developing small molecules that bind individual domains to inhibit protein function.
16 Understanding the true “on” and “off” target contributions to pharmacological activity is
17 one of the pillars of target validation¹, but can be difficult to realize when the protein in
18 question shares homologous domains with other proteins. This is especially true of
19 epigenetic protein families that mediate transcriptional signaling through the catalytic or
20 scaffolding function of their structural domains², many of which are difficult to target
21 selectively with small molecules because of their conserved binding sites³. In this issue,
22 Hohmann *et al.*⁴ describe an elegant approach that involves swapping the targeted
23 domain with one from a structurally homologous family member that does not bind the
24 probe, to confer probe resistance and validate the relevant cellular target.

25
26 Epigenetic effectors are multi-domain proteins that catalyze installment, removal or
27 recognition of posttranslational modifications of amino acids on histones amongst other
28 substrates. Many epigenetic proteins function as part of large complexes that bind to
29 promoters, enhancers and super-enhancer regions of the genome, remodeling chromatin
30 and regulating transcription of genes that can sustain growth and proliferation of cancer
31 cells⁵. Amongst these are the chromatin remodelers switch/sucrose non-fermentable
32 (SWI/SNF), multisubunit enzymes that utilize the energy derived from ATP hydrolysis to
33 open up chromatin and activate transcription⁶. Identifying the most relevant target
34 protein in a given disease context is an important first step.

35
36 In the study by Hohmann *et al.* Brd9, which is a subunit of a mammalian SWI/SNF complex
37 (also known as BAF)⁶, was found to be required for the growth of acute myeloid leukemia
38 (AML) cells. Brd9 supports leukemic maintenance by sustaining *Myc* expression via its
39 essential bromodomain - the reader domain of lysine acetylation. These observations
40 provided convincing genetic rationale for chemical probe discovery effort, and
41 nanomolar-potency inhibitors targeting the Brd9 bromodomain were developed by
42 fragment-based drug design, achieving >1,000 fold selectivity against the main potential
43 off-target bromodomain of the BET protein Brd4⁷. Inhibitor treatment led to the release,
44 albeit partial, of the entire BAF complex from chromatin, and phenocopied the cellular
45 effect and transcriptional response of Brd9 knockdown (Fig. 1a). Crucially, an engineered
46 Brd9 variant in which the bromodomain of Brd9 was substituted by the first bromodomain
47 of Brd4 was devised. This “domain-swap” allele *BRD9-BET* proved insensitive to chemical
48 inhibition by chemical probe BI-7273 but could substitute the wild-type function,

providing a powerful genetic tool to validate Brd9 as the relevant cellular target, and demonstrating the superior on-target effect of BI-7273 over other Brd9 probes⁴ (**Fig. 1b**).

The most compelling finding of this study for chemical biology is the replacement of entire functional domains as a successful strategy to generate inhibitor-resistant alleles. This new approach extends on the use of naturally emerging or engineered drug-resistant mutations, which have proven of broad utility in the protein kinase space but have yet to be established with epigenetic targets. With success, chemical probes have been rendered exquisitely selective by chemical genetic approaches such as the “bump and hole”⁸, however for this to be generally applicable a pan-selective tool compound is required and single-point mutations have to be designed that do not significantly alter protein function, leaving the need for the mutant to be complemented by adequate synthetic modifications of the probe itself. The discovery that the *BRD9-BET* allele retained sufficient functionality in AML despite the evolutionary distance was unexpected, in part because it is known that bromodomains have different recognition specificities to histone peptides across the family². This observation suggests context- and loci- dependent specificities of chromatin occupancy of epigenetic proteins than might not be revealed using isolated domains and truncated histone peptides as substrates. The new findings bear also implications for targeting cancer epigenetics. The biological activities of the reported probes provide important benchmark for non-BET bromodomain inhibitors, which have previously proved unexceptional in cells. For example PFI-3, a chemical probe for the bromodomain of Brg1 (also known as *Smarca4*, another BAF subunit), failed to display anti-tumoral activity in cancer cells sensitive to Brg1 knockdown⁹. This highlights that distinct domain interactions may have different susceptibilities to pharmacological target modulation, despite being present within the same complex.

How general could the domain-exchange approach be? The data presented⁴ on the methyltransferase EZH2, a prominent cancer target also found within a multiprotein histone-modifying complex⁶, and its SET domain-targeting probe GSK126, encourages optimism and a broadly applicable appeal, at least in the context of epigenetic domains. CRISPR-Cas9 screens are increasingly being developed to reveal cancer dependencies of epigenetic domains¹⁰, and it might be possible in many cases to identify paralogous domains that once swapped can be expressed in a functionally silent manner, without exhibiting dominant-negative effects on the endogenous protein. Ultimately, genetic engineering of homozygous domain swap knock-ins could surpass the need for transducing and selecting for exogenous alleles, not only improving target validation of existing probes but also opening new doors to the development of target-sensitive phenotypic screens in disease-relevant models. So switch domain, and validate your target!

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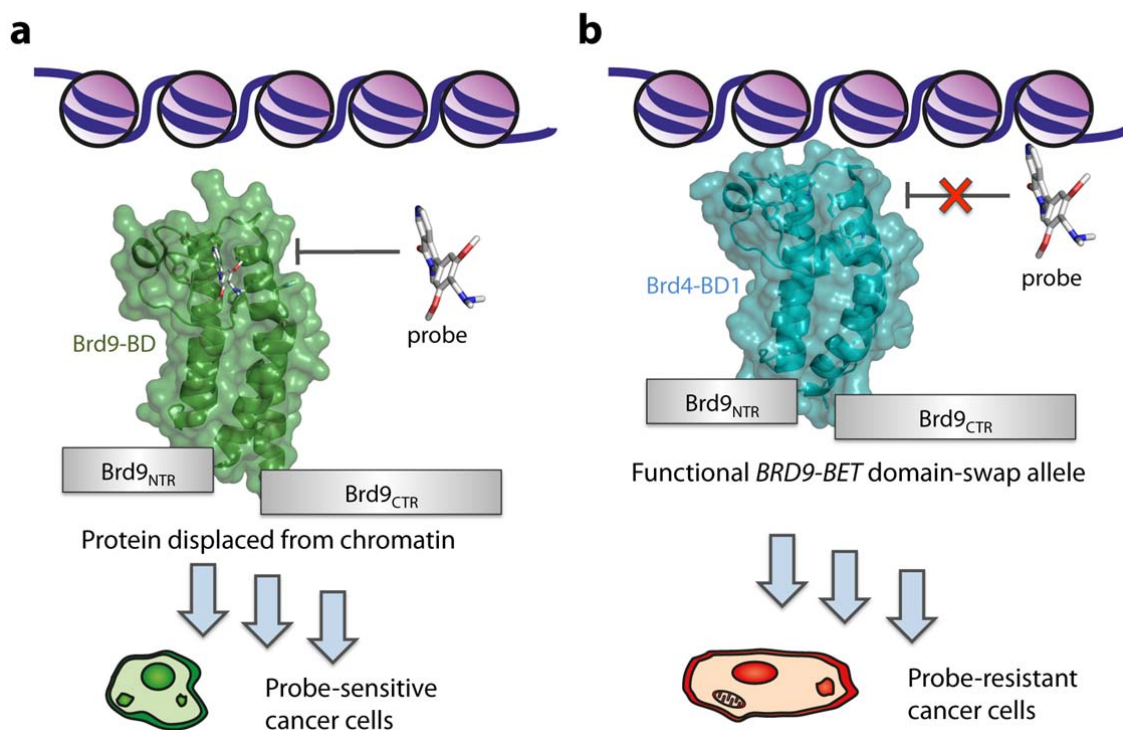


Figure 1.

Domain-swap to assess chemical probe on-target effects. (a) Probe binds to the target domain (BD, bromodomain), leading to pharmacological effect in cancer cells. (b) Swapping that target domain for one from a different protein that does not bind the probe leads to probe-resistant cells, establishing the probe's on-target effects. In each case the remainder of the target protein remains unaltered (NTR, N-terminal region; CTR, C-terminal region).

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